Acute Myeloid Leukemia and Acute Leukemias of Ambiguous Lineage

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Acute Myeloid Leukemia (AML)

- Clonal proliferation of myeloid precursors with a reduced capacity to differentiate into more mature cellular elements.
  - accumulation of blasts in the bone marrow, peripheral blood, and occasionally in other tissues
    - reduction in the production of normal red blood cells, platelets, and mature granulocytes
- Present with symptoms related to complications of pancytopenia
  - anemia, neutropenia, and thrombocytopenia
  - Infections, general fatigue, pallor

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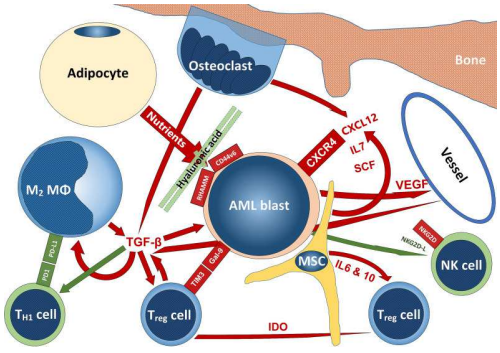
Morphology

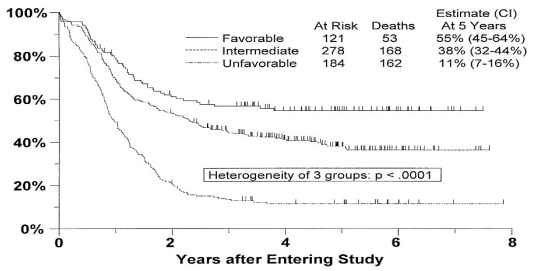
- Morphology remains the key step to directing other studies
- Assess blast cell percentage
  - Manual count still required
    - Flow cytometry blast counts may give erroneous results
    - Immunohistochemical stains helpful in fibrotic samples
- Assess specific blast cell features
  - Blast equivalents include abnormal promyelocytes and immature monocytic cells (monoblasts/promonocytes), megakaryoblasts, erythroblasts
- Assess non-blast cell lines
  - Presence of abnormal eosinophils, megakaryocytes, etc
  - Identification of increased basophils and eosinophils
  - Detection of multilineage dysplasia



Cytogenetics in AML

- Diagnostic karyotype has been found to predict response to induction therapy, relapse risk and overall survival
  - biologically distinct subsets of disease
- Categorizes patients into favorable, intermediate and unfavorable risk groups
- Complex karyotype is defined as more than 3 unrelated cytogenetic abnormalities

Southwest Oncology Group/Eastern Cooperative Oncology Group study



Marilyn L. Slovak et al. Blood 2000;96:4075-4083

Cytogenetic Risk Groups

Low	**t(8;21)**	**High**	**Complex (>3) abnormalities**
	inv(16)/t(16;16)		**-7**
	t(15;17)		**inv(3q)**
			del(9q) without t(8;21)
Intermediate	**Normal karyotype**		**11q23, 17p, 20q or 21q abnormalities**
	Single abnormalities		
	+8		**t(9;22)**
	+11		**t(6;9)**
	-Y		**+13**
	12p abnormalities		

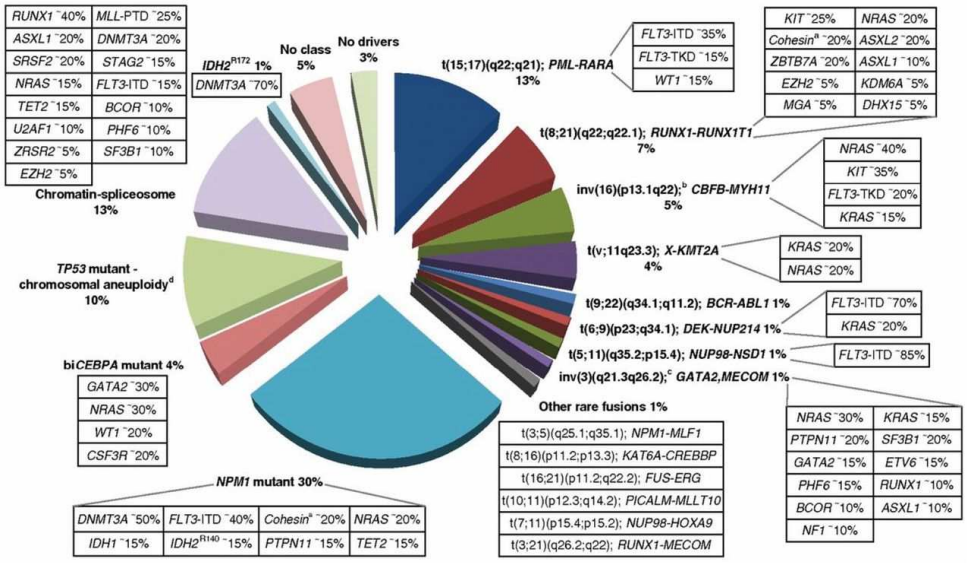
Complex karyotype ≥3 or ≥5

AML with recurrent genetic abnormalities

- AML with t(8;21)(q22;q22)(RUNX1-RUNX1T1)
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)(CBFB-MYH11)
- APL with t(15;17)q24.1;q21.1)(PML-RARA)
- AML with t(6;9(p23;q34)(DEK-NUP214)
- AML with inv(3)(q21q26.2) or t(3;3)(p13;q13)(RBM15-MKL1)
- AML (megakaryoblastic) with t(1;22)(p13;q13)(RBM15-MKL1)

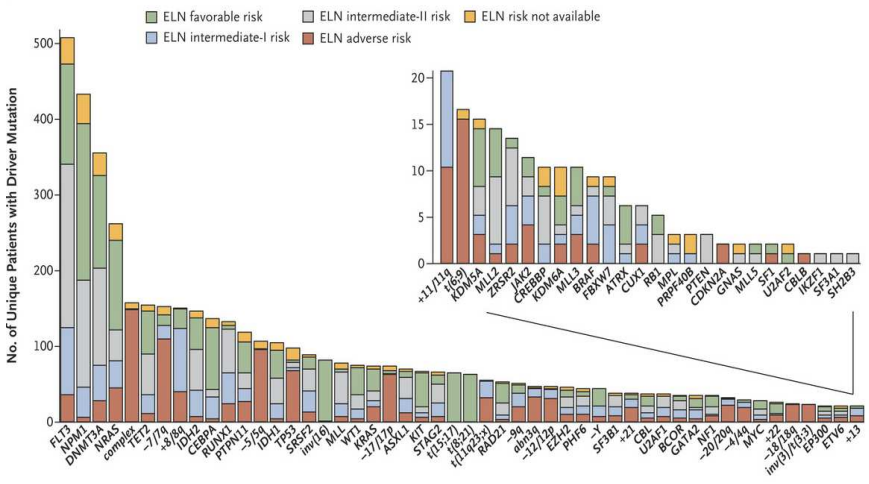
- Provisional entity: AML with mutated *NPM1*
- Provisional entity: AML with mutated *CEBPA*

Molecular classes and recurrent gene mutations in AML



J Natl Compr Canc Netw 2018 May;16(5S):656-659.

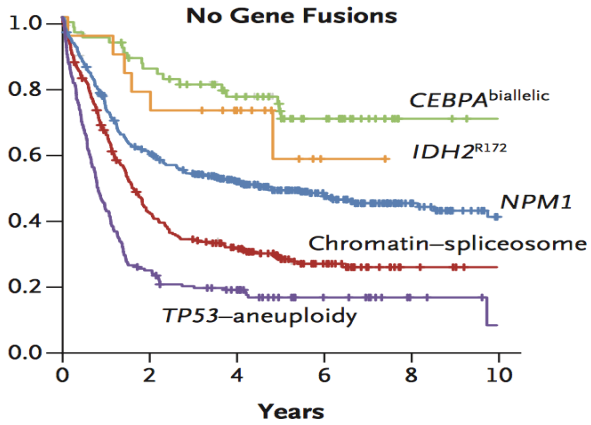
Landscape of Driver Mutations in AML

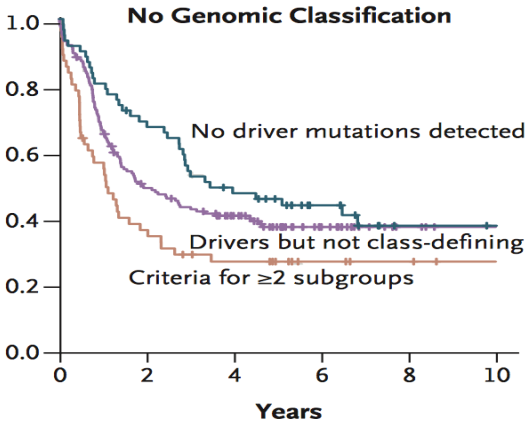


- 1540 patients in three prospective trials of intensive therapy
- 5234 driver mutations across 76 genes or genomic regions
- 2 or more drivers identified in 86% of the patients

N Engl J Med. 2016 Jun 9;374(23):2209-2221

Genomic Classification in AML





Chromatin-spliceosome group

SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1, STAG2, BCOR, MLL, EZH2, RUNX1 and *PHF6*

N Engl J Med. 2016 Jun 9;374(23):2209-2221

LeukemiaNet Prognostic Genetic Categories

Genetic Group	Subsets
Favorable	•t(8;21)(q22;q22); *RUNX1-RUNX1T1* •inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* •Mutated *NPM1* without *FLT3*-ITD (normal karyotype) •Mutated *CEBPA* (normal karyotype)
Intermediate-I	•Mutated *NPM1* and *FLT3*-ITD (normal karyotype) •Wild-type *NPM1* and *FLT3*-ITD (normal karyotype) •Wild-type *NPM1* without *FLT3*-ITD (normal karyotype)
Intermediate-II	•t(9;11)(p22;q23); *MLLT3-MLL* •Cytogenetic abnormalities not classified as favorable or adverse
Adverse	•inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1* •t(6;9)(p23;q34); *DEK-NUP214* •t(v;11)(v;q23); *MLL* rearranged •−5 or del(5q); −7; abnl(17p); complex karyotype

2017 ELN risk stratification by genetics

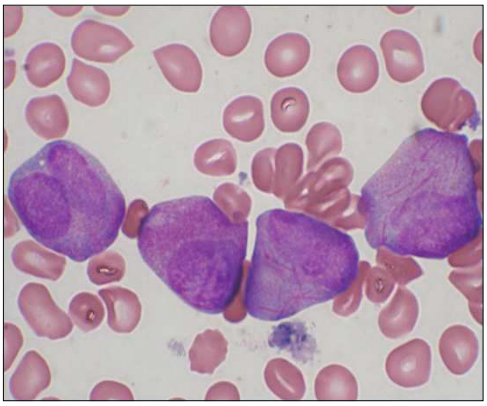
Risk category*	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low†}
	Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high†}
	Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low†} (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*‡
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214*
	t(v;11q23.3); *KMT2A* rearranged
	t(9;22)(q34.1;q11.2); *BCR-ABL1*
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)*

Blood (2017) 129 (4): 424–447

Acute Promyelocytic Leukemia

Diagnostic emergency

- 5-8% of all AML cases
- Hypergranular APL – 60-70% presents with low white blood cell count
  - Hypogranular variant presents with leukocytosis
- Rare cases do not resemble either hypergranular or microgranular APL
- Minority of cases cryptic (negative) by cytogenetics and FISH, but PCR positive
- Early ATRA essential to reduce risk of hemorrhage



Translocations in Acute Promyelocytic Leukemia

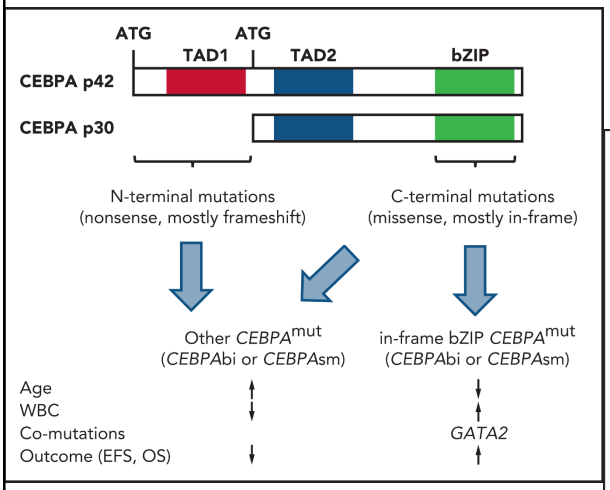
Cytogenetics	Fusion Proteins	Frequency	Response to All-*trans* Retinoic Acid	Prognosis	Unique Features
t(15;17)(q22;q21)	PML/RARA	98%	Responsive	Favorable	None
t(11;17)(q23;q21)	ZBTB16/RARA	0.8%	Resistant	Worse prognosis	Regular nucleus, fine or absent granules, increased CD56 expression
t(5;17)(q35;q21)	NPM/RARA	Rare	Responsive, but higher risk of relapse	Favorable, but higher risk of relapse	Pediatric patients
t(11;17)(q13;q21)	NUMA/RARA	Rare	Responsive	Favorable	None
der(17)	STAT5B/RARA	Rare	Resistant	Worse prognosis	None
der(17)	PRKAR1a/RARA	Rare	Responsive	Favorable	None
t(X;17)(p11;q12)	BCOR/RARA	Rare	Responsive	Favorable	None
t(4;17)(q12;q21)	FIP1L1/RARA	Rare	Responsive	Favorable	None

majority are similar morphologically to classic APL

- except ZBTB16-RARA t(11;17) APL, which has distinct cytologic features
- hypogranular pelgeroid neutrophils

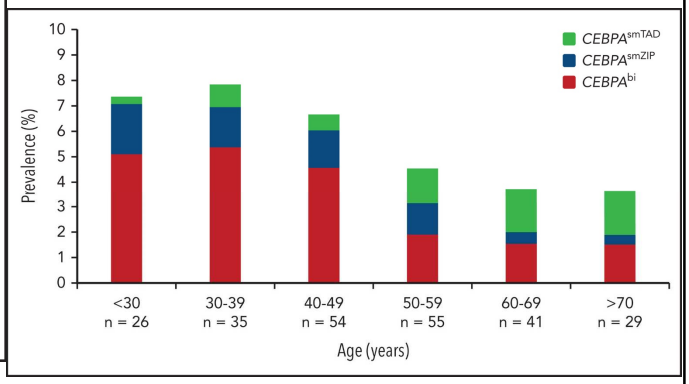
Arch Pathol Lab Med 2015 Oct;139(10):1308-13

CEBPA mutations in AML: site matters



Taube et al: 4708 patients with AML fragment analysis/targeted sequencing

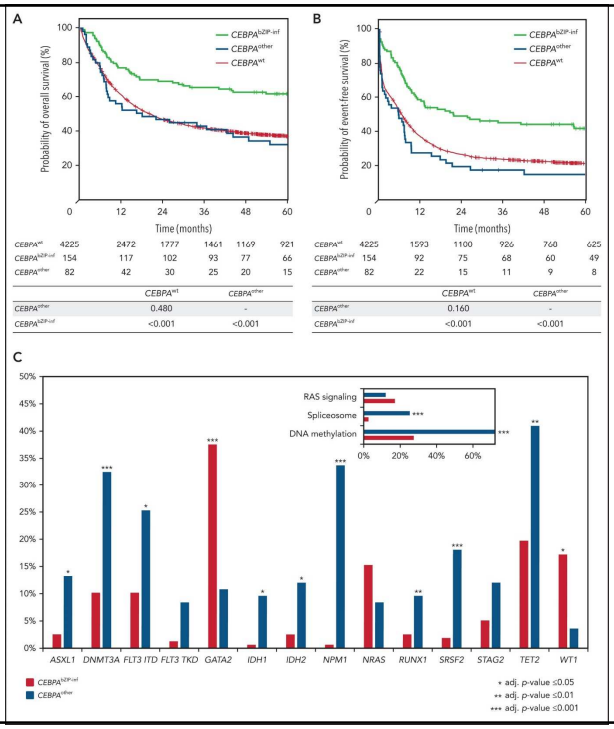
- 240 *CEBPA* mutations (5%) – 131 CEBPAbi, 109 sm (60 TAD (25%) and 49 bZIP (20%))
- Age distribution of the 240 *CEBPA* mutations



Blood (2022) 139 (1): 6–7

Blood (2022) 139 (1): 87–103

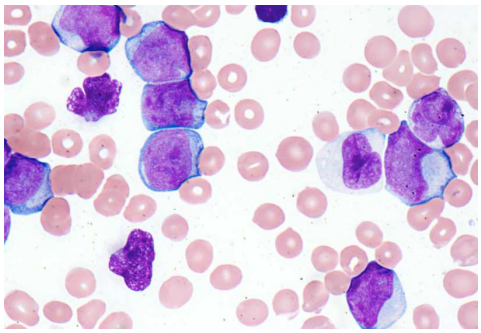
- CEBPAsmbZIP- and CEBPAbi-mutant AML share clinical and mutational characteristics and are distinct from CEBPAsmTAD-mutant AML
- Only in-frame mutations in *CEBPA*-bZIP are associated with favorable clinical response in both monoallelic and biallelic constellations
  - Previously undefined prognostic role



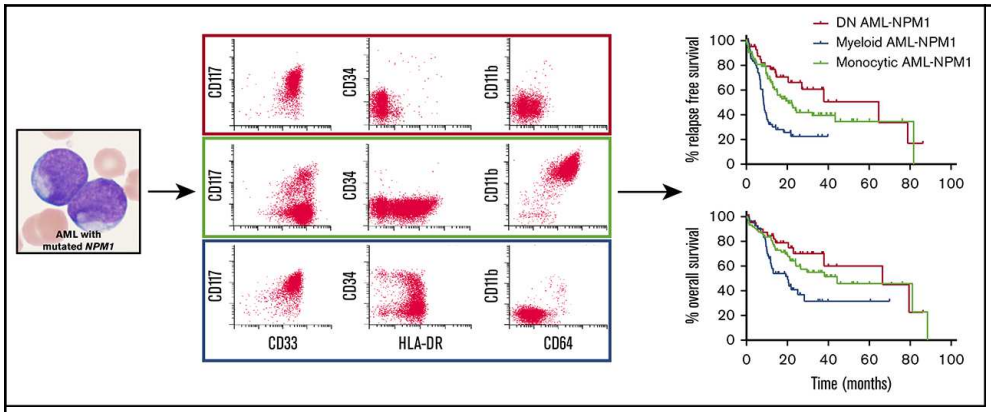
Blood (2022) 139 (1): 87–103.

AML with *NPM1* mutations

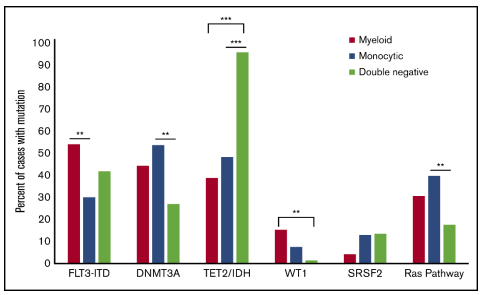
- One of the most common mutations in normal karyotype AML
- Multilineage dysplasia in this setting has been shown to not have clinical significance
  - Presence of NPM1 mutation trumps multilineage dysplasia
- Secondary AML cases (arising from MDS, MPN, therapy related) lack favorable prognosis of de novo AML

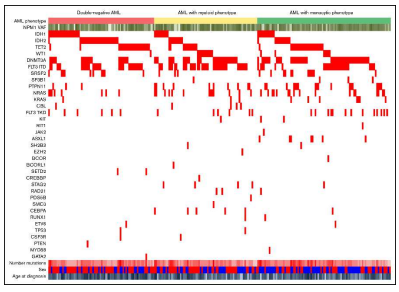


Diaz-Beya M et al Blood 2010;116: 6147-8



Blast phenotype and comutations in acute myeloid leukemia with mutated *NPM1* influen ce disease biology and outcome



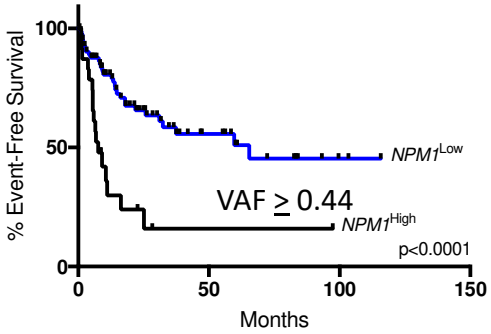


Blast phenotype is an independent predictor of outcome in NPM1-mutated AML.

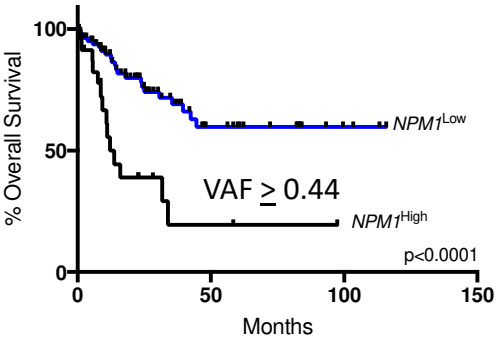
Patients with a DN blast phenotype (lacking CD34 and HLA-DR expression) harbor TET2/IDH mutations and show superior outcomes.

Blood Adv (2019) 3 (21): 3322–3

High *NPM1* mutant allele burden at diagnosis predicts unfavorable outcomes in de novo AML



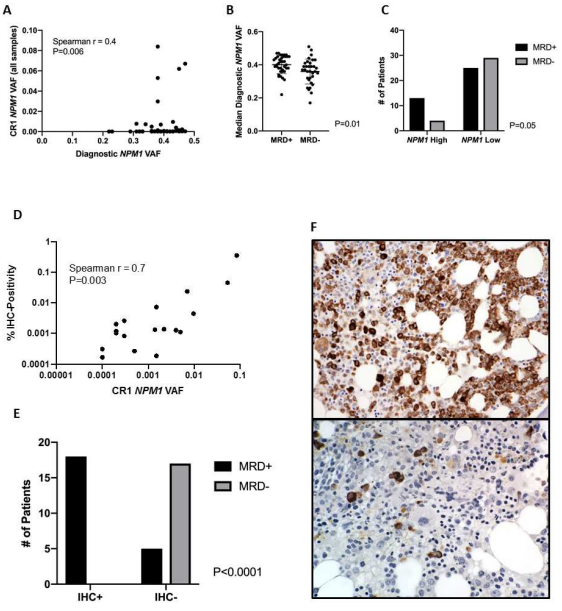
7.5 vs. 65.4 mos., p<0.0001

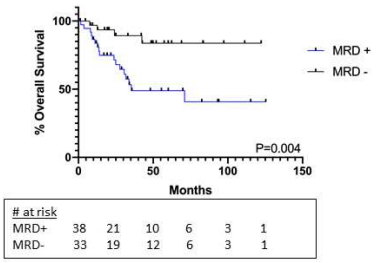


12.1 mos. vs. not reached., p<0.0001

Blood. 2018 Jun 21;131(25):2816-2825

Correlations between molecular MRD at CR1 and diagnostic *NPM1* VAF and mutant NPM1 protein (NPM1c) expression by IHC at CR1





# at risk						
MRD+	38	21	10	6	3	1
MRD-	33	19	12	6	3	1

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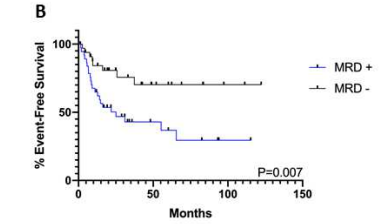


# at risk						
MRD+	38	16	8	5	2	1
MRD-	33	17	10	6	3	1

Am J Hematol. 2019 Aug;94(8):921-928

Characteristics of NPM1-mutated "non-AML" cases

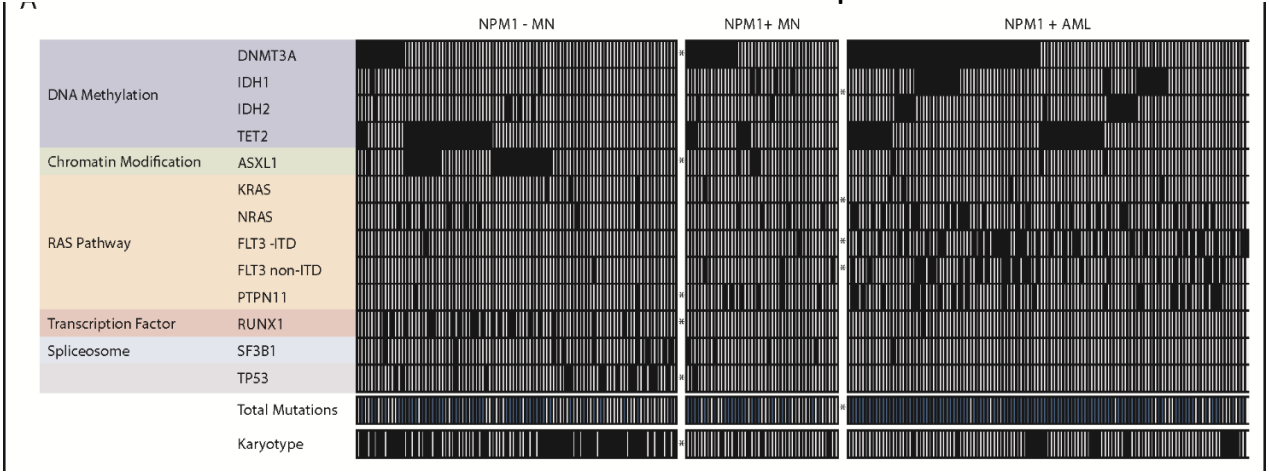
- Multi-institutional study of 45 cases with <20% blasts, but *NPM1* mutation
- Assessed clinical features, co-mutations, and patient outcome

	NPM1- MN (n=95)	*NPM1*+ MN (n=45)	*NPM1*+ AML (n=119)
Patient Characteristics			
Median of age (range), years	68 (38-84)*	63 (36-96)	61 (15-85)
M:F	1.9	1.0	0.75
Clinical Parameters			
Hemoglobin (g/dL), median (range)	9.7 (4.8-15.9)	9.0 (6.1-12.7)	9.0 (5.7-15)
WBC (K/μL), median (range)	3.5 (0.6-69.4)	3.3 (1.2-225)	21 (0.69-340)*
Platelet count (K/μL), median (range)	84 (15-808)	79 (15-607)	72 (10-356)
Median of BM cellularity, % (range)	70 (10-95)	80 (10-100)	90 (30-98)*
Median of BM Blasts, % (range)	8 (1-18)	10 (1-19)	73 (21-96)*
Diagnosis			
MDS non-EB, n (%)	5 (5)	2 (4)	n/a
MDS-EB, n (%)	55 (58)	24 (53)	n/a
CMML, n (%)	16 (17)	9 (20)	n/a
MDS/MPN (non-CMML), n (%)	8 (8)	5 (11)	n/a
t-MN, n (%)	11 (12)	5 (11)	n/a
AML, n (%)	n/a	n/a	119 (100)
IPSS-R scores (MDS cases only), median (range)	5.0 (1.0-10.0)	5.0 (1.5-7.0)	n/a
Outcome			
Median Follow-up Time, months (range)	19.4 (0.3-57)	10 (0.07-70)	24 (0.13-125)
Alive at last follow-up, n (%)	53 (56)	29 (64)	67 (56)
Progression to AML, n (%)	30 (32)	20 (44)	n/a
Median time to progression, months (range)	6.3 (1.7-43)	5.2 (0.4-17.5)	n/a
Received up-front HMA therapy, n (%)	55 (58)	33 (73)	5 (4)
Received up-front induction chemotherapy, n (%)	0 (0)	3 (7)	113 (95)
Received SCT at any time, n (%)	44 (46)	19 (42)	67 (56)

Patel S Blood Advances 2019;3:1540

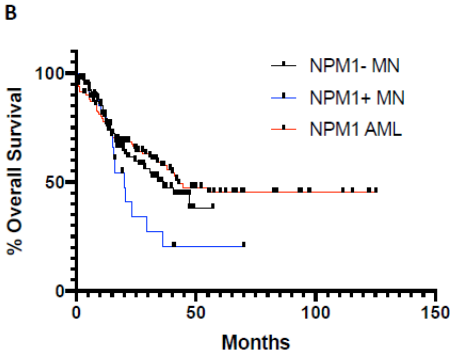
NPM1 mutation in myeloid neoplasms with <20% blasts is similar to NPM1+ AML

Mutation heatmap



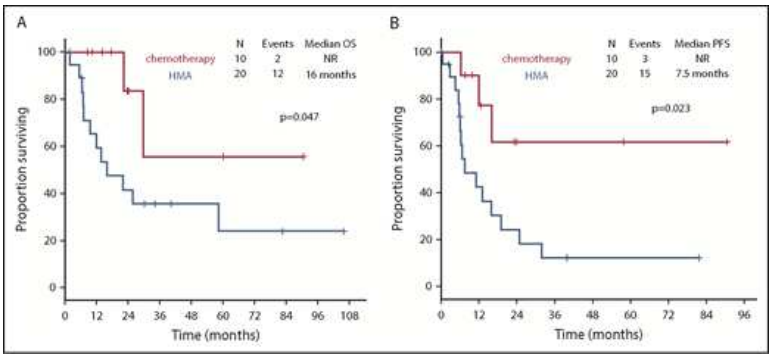
Patel S Blood Advances 2019;3:1540

NPM1-mutated non-AML patients appear to do poorly if treated as MDS



- 3 patients treated with up-front induction did not progress to AML, 2 still alive.
- 5/5 untreated patients progressed to AML in a median of 3 months

Outcome of *NPM1*-mutated non-AML patients based on induction versus HMA (retrospective)

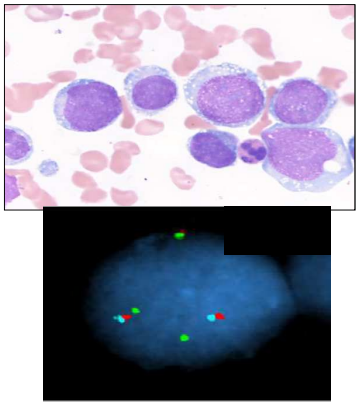


Most patients were classified as **MDS with excess blasts** (MDS-EB; 19/31, 61%), 3 (10%) being classified as MDS-EB-1 and 16 (52%) being classified MDS-EB-2

Montalban-Bravo G Blood Advances 2019;3:922, Patel S Blood Advances 2019;3:1540

AML with *BCR-ABL1*

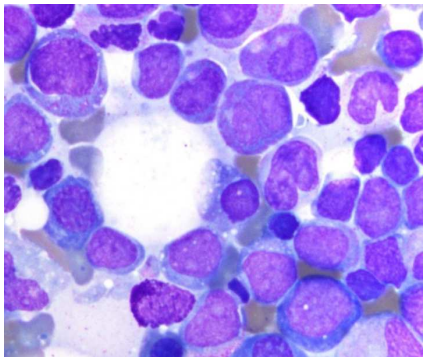
- De no AML
  - No evidence (before or after therapy) of chronic myeloid leukemia
  - <1% of all AMLs
- Most cases have p210 fusion, with b2a2 and b3a2 fusions being next most common.
  - A minority of reported cases with p190 transcripts
- Exclude cases that meet criteria for MPAL, therapy related neoplasms or other AML cases with recurrent genetic abnormalities
- Deletion of antigen receptors (IGH, TCR), *IKZF1* and/or *CDKN2A* may support a diagnosis of de novo disease
- Patients may benefit from targeted (TKI) therapy



Soupir CP, et al. Am J Clin Pathol 127:642, 2007
Konoplev S, et al. Leuk Lymphoma 54:138, 2013
Nacheva EP, et al. Br J Haematol 161:541, 2013

AML with mutated *RUNX1*

- Gene located at 21q22
  - Encodes the alpha subunit of the core binding factor
- Present in 12.5-13.2% of AML
  - More frequent in older male patients
  - Frequent prior history of MDS, or prior exposure to radiation
  - Wide morphologic spectrum
- Frequently associated *KMT2A*-PTD, *IDH1, IDH2* or *ASXL1* mutations
  - Rare *CEBPA* or *NPM1* mutations
- Poor response to therapy with shortened survival
- Germline mutations should be evaluated
- Cases arising from MDS will still be called AML- MRC
- Cases with prior therapy will still be therapy- related AML



Tang et al. Blood 114:5352, 2009
Mendler et al. J Clin Oncol 30:3109, 2012

AML with Myelodysplasia related changes

- Detection of multilineage dysplasia
  - Two non-blast cell lines must show dysplasia in at least 50% of cells
- MDS-related cytogenetic abnormalities or prior MDS/MPN
- Absence of the specific genetic abnormalities of AML with recurrent genetic abnormalities
- Absence of prior history of therapy
- Cases with dysplasia and *NPM1* or *CEBPA* mutations are classified as AML with RGA
- Deletion 9q

  association with t(8;21),
  frequently occurs in AML with NPM1 and biallelic CEBPA mutations

Complex karyotype (≥3 abnormalities)

Unbalanced abnormalities

- Loss of chromosome 7 or del(7q)
- del(5q) or t(5q)
- Isochromosome 17q or t(17p)
- Loss of chromosome 13 or del(13q)
- del(11q)
- del(12p) or t(12p)
- idic(X)(q13)

Balanced abnormalities

- t(11;16)(q23.3;p13.3)
- t(3;21)(q26.2;q22.1)
- t(1;3)(p36.3;q21.2)
- t(2;11)(p21;q23.3)
- t(5;12)(q32;p13.2)
- t(5;7)(q32;q11.2)
- t(5;17)(q32;p13.2)
- t(5;10)(q32;q21)
- t(3;5)(q25.3;q35.1)

AML with t(6:9)(p23;q34); *DEK-NUP214*

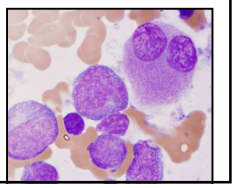
Frequently associated with erythroid hyperplasia and multilineage dyplasia

Basophilia common

AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*

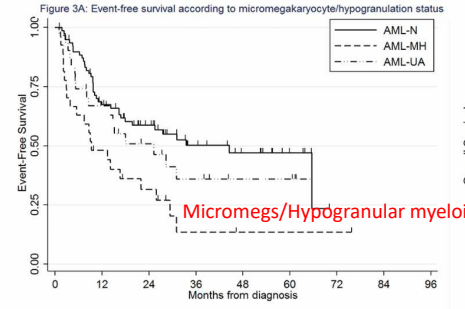
Thrombocytosis

Multilineage dysplasia with atypical small megakaryocytes

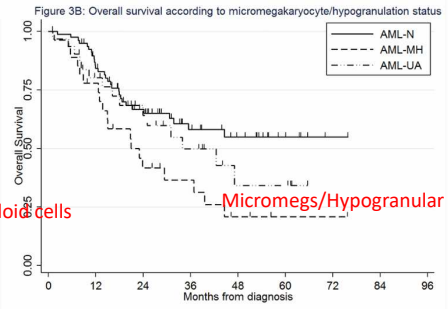


Micromegakaryocytes and Hypogranulated myeloid cells

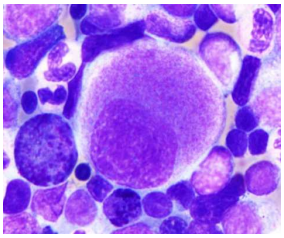
independently associated with shorter OS/EFS in multivariable

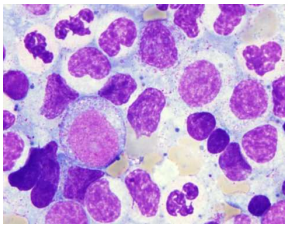


Micromegs/Hypogranular myeloid cells



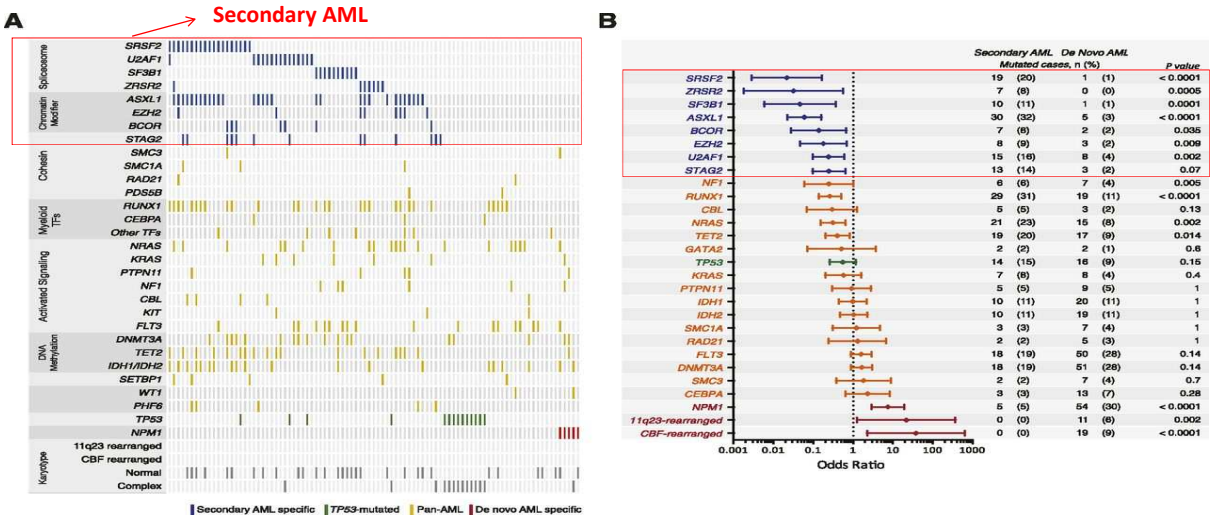
Micromegs/Hypogranular myeloid cells





Mod Pathology 2015 28(7):965-76

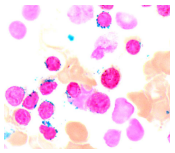
Using mutations in defining AML ontogeny

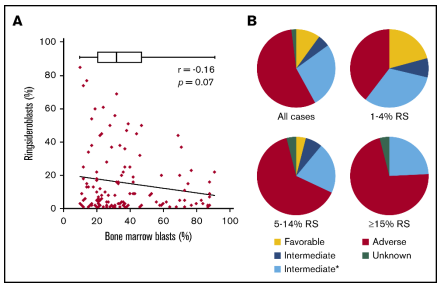


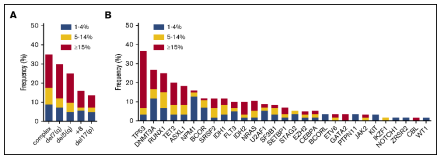
Secondary AML=AML arising from MDS or CMML

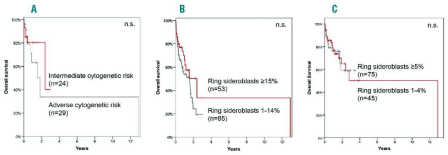
R. Coleman Lindsley et al. Blood 2015;125:1367-1376

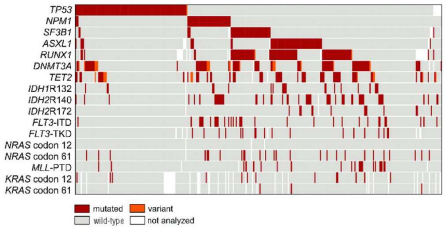
Ring sideroblasts in AML are associated with adverse risk characteristics and have a distinct gene expression pattern











Blood Adv 2019 Oct 22;3(20):3111-3122

Haematologica 2017;102:e125-e128

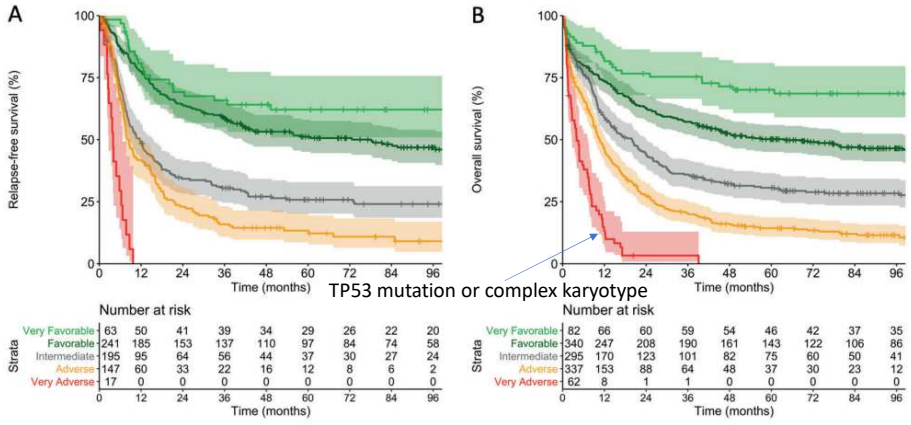
TP53 mutation in AML

- 10-15% of AML cases overall
- Much higher prevalence in therapy-related AML
  - Expansion of *TP53*-mutated clonal hematopoiesis fostered by effects of chemotherapy on bone marrow microenvironment
- 50-80% of complex karyotype AML has *TP53* mutation,
  - ~90% of *TP53*-mutated AML has complex karyotype
    - Typically losses of 5q, 7q, and or 17p, and monosomal karyotype
  - *TP53* mutation less frequent in complex karyotypes lacking 5q-/7q-/17p-
- Present at the time of initial diagnosis in most cases (85%) and stable during disease evolution or followup

Hou H-A et al. Blood Cancer J 2015;5:e331, Mrozek K et al. Leukemia 2019;33:1620, Wong TN et al. Nature 2015;518:552, Papaemmanuil et al., NEJM 2016;374:2209

TP53 mutation confers very poor prognosis in AML

- Considered a high-risk mutation in ELN scheme, but *TP53*-mutated patients do even worse than other high-risk patients



Herold T et al. Leukemia 2020;34:3161.

Outcome studies in *TP53*-mutated AML

- Intensive therapy does not appear to improve outcome over lower-intensity therapies, even in younger patients with high blast counts.
- *TP53*-mutated AML also associated with poor response to decitabine/venetoclax (median OS of 5.2 months, compared to 19.4 m for *TP53*wt AML) and CPX-351 (even compared to other ELN high-risk)
- Outcomes poor after SCT
- *TP53* mutations developing secondarily at AML relapse (~15% of patients) are also associated with short survival (median OS of 4.6 months)
- Co-mutations and mutant allele status (multi-hit versus single *TP53* mutation) do not appear to influence prognosis

Alwash Y et al. Am J Hematol 2021 (Epub), Chiche E et al. Blood Adv 2021;5:176, Kim K et al. Cancer 2021 (Epub), Bewersdorf JP et al. Leuk Lymphom 2020;61:2180, Middeke JM et al. BJH 2016;172:914-22, Valk et al, Unpublished data.

How to identify *TP53* multi-hit?

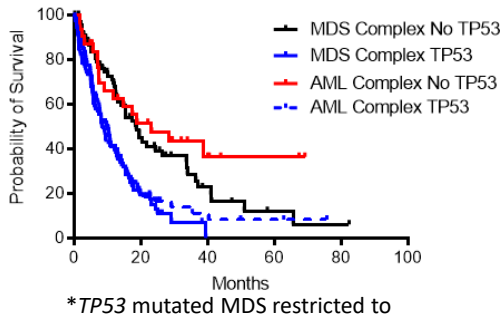
- Multi-hit signifies loss of wild-type allele through
  - Presence of >1 *TP53* mutation (i.e. both alleles mutated) (24%)
  - Presence of 1 mutation with deletion of second allele (usually identified by karyotype)(22%)
  - Presence of 1 mutation with copy-neutral LOH (21%)
    - Requires copy-number analysis that is not performed in most standard clinical NGS panels, but can be approximated by high *TP53* VAF >60%)

Bernard E et al. Nat Med 2020;26:1549

Do *TP53*-mutated AML and MDS represent a single entity with shared biology and dismal prognosis?

299 complex karyotype AML and MDS cases (0-94% blasts)

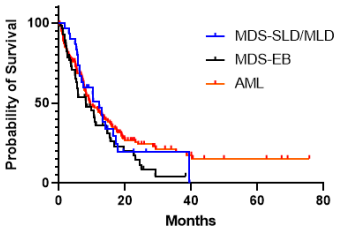
MDS and AML OS by TP53 status



**TP53* mutated MDS restricted to multi-hit *TP53* (64% of cases)

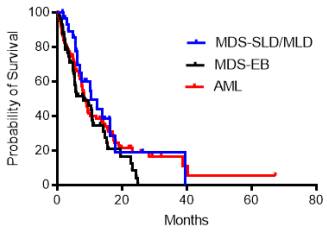
- No influence of therapy-relatedness or monosomal karyotype on outcome in AML, MDS, or combined cohorts

MDS and AML OS by TP53 status



p=0.21

MDS and AML OS by TP53 status
Censored at time of SCT

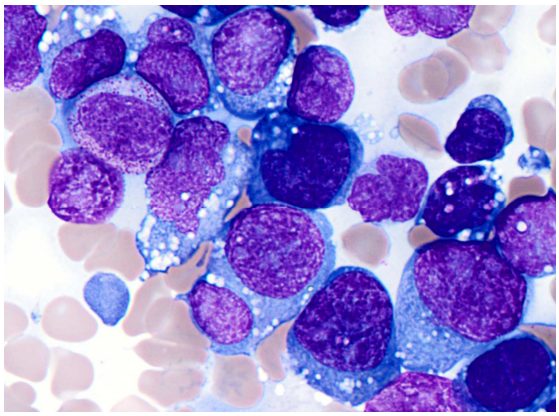


p=0.23

Weinberg O et all Blood Adv 2022 Jan 24

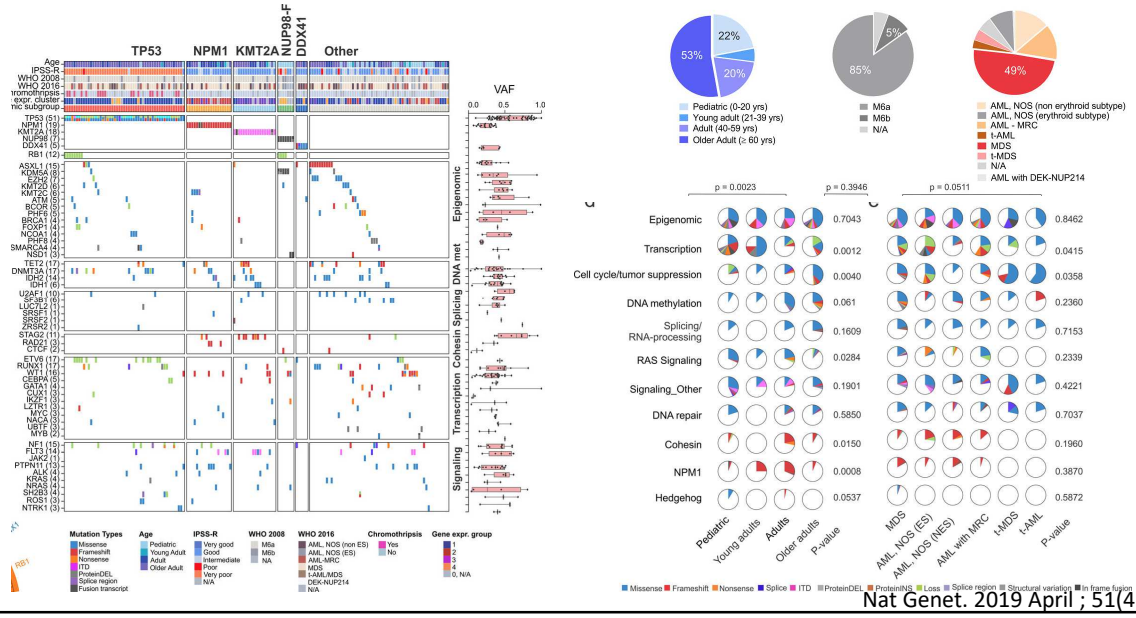
Acute erythroid leukemia

- Proliferation of immature cells committed exclusively to the erythroid lineage
  - > 80% of the bone marrow cells are erythroid, with >30% proerythroblasts),
- Often occurs as progression from prior MDS or therapy related disease
  - Therapy related neoplasm
- Blasts express CD117, CD71, CD36, CD235
- AML M6a (myeloid/erythroid) reclassified based on total marrow blast count -> MDS or AML



Genomic Subtyping of Acute Erythroleukemia

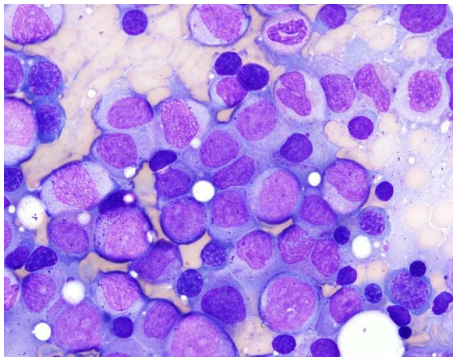
159 cases included 35 pediatric cases (0–20 years, 22%), 8 young adults (21–39 years, 5%), 32 adults (40–59 years, 20%), and 84 older adults (≥ 60 years, 53%)



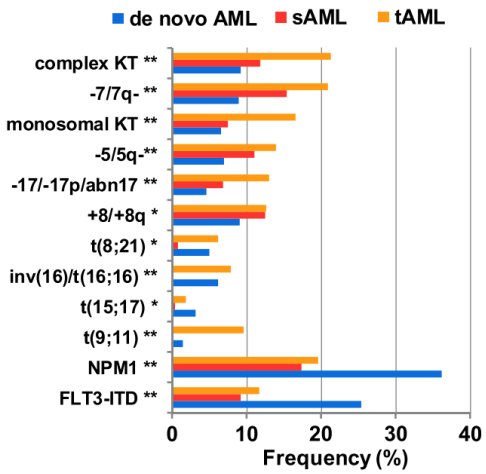
Nat Genet. 2019 April ; 51(4): 694–704

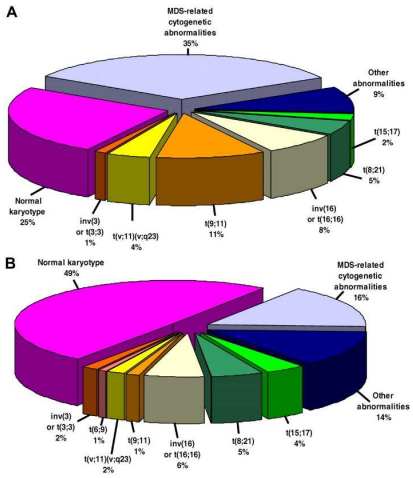
Therapy related myeloid neoplasms

- Patients who develop myeloid neoplasms following cytotoxic therapy
  - 70% treated for solid malignancy and 30% for hematologic malignancy
  - common occurs 5–10 years after exposure to alkylating agents and/or ionizing radiation
  - 1–5years follows treatment with agents that interact with DNA topoisomerase II (topoisomerase II inhibitors).
- AML and MDS grouped together
- May have recurring cytogenetic abnormalities that impact prognosis and should be noted in diagnosis
- May occur after therapy for another AML type



Genetics of t-AML



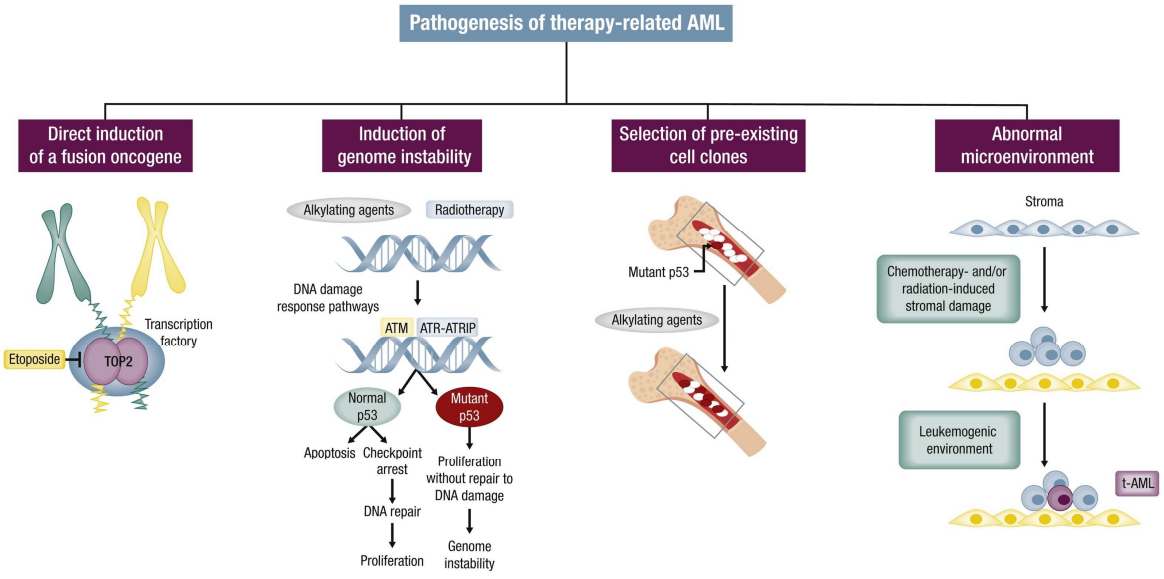


t-AML

De novo AML

Hematology Am Soc Hematol Educ Program 2016 Dec 2;2016(1):24-32.

Blood (2011) 117 (7): 2137–2145.



Crit Rev Oncol Hematol 2022 Jan 28;103607

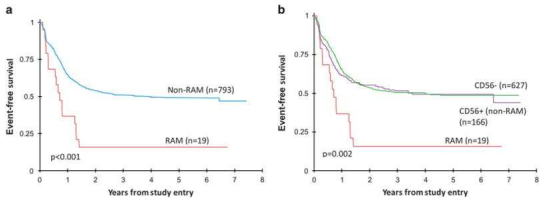
Inherited cancer susceptibility

- German AML Study Group (AMLSG) published data on the latency from diagnosis of the primary malignancy to t-AML.
- Seven percent of a cohort of 2835 patients with AML developed t-AML after chemotherapy and/or radiotherapy for the primary malignancy, with a median latency of ~4 years.
- Interestingly, 3% developed AML after a diagnosis of an independent malignancy that had never been treated with chemotherapy or radiotherapy.
  - Compared with t-AML patients, these patients more often had prostate cancer (23% vs 9%), bladder cancer (9% vs 1%), and renal cell carcinoma (9% vs 2%), but less often had breast cancer (10% vs 52%).
  - AML developed in these patients with no history of chemotherapy or radiotherapy, with a median latency of 5 years, which is similar to that of patients with t-AML

Blood. 2011 Feb 17; 117(7):2137-45.

AML with RAM phenotype

- In 2016, Brodersen et al reported an immunophenotype that is associated with poor prognosis in pediatric patients
- Assessed 821 new pediatric AML immunophenotypes
  - Excluded Down syndrome and APML patients
- Identified 19 patients with 4 unique immunophenotypic features
  - Bright CD56 expression
  - Dim-to-negative CD45
  - Dim-to-negative CD38
  - Absent HLA-DR
  - RAM patients had intermediate-risk cytogenetics and lacked molecular risk features (*FLT3-ITD, CEBPA* or *NPM1*)

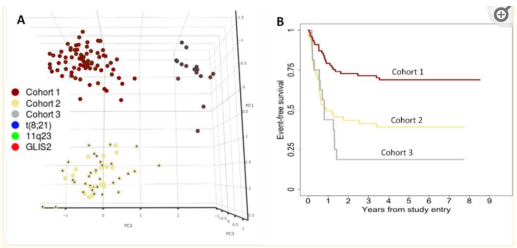


Leukemia 2016 Oct; 30(10): 2077–2080.

Deciphering the Significance of CD56 Expression in Pediatric Acute Myeloid Leukemia: a Report from the Children's Oncology Group

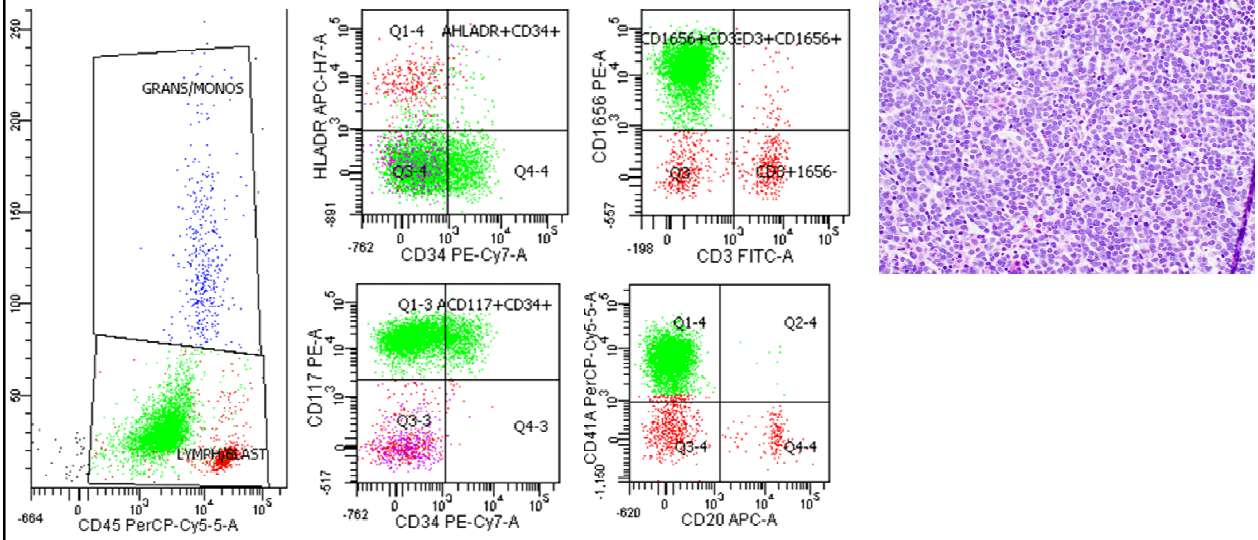
769 newly diagnosed pediatric patients with de novo AML enrolled in AAML0531

Cohort	Immunophenotype summary	Prevalent Cytogenetic/molecular (Percent out of total patients in cohort)	Risk classification defined by AML0531 protocol	Five-year EFS % (±2SD)
1 *N*=77	• Increased CD56 • CD34 and HLA-DR positive • CD38 intermediate	Prevalence of t(8;21) (72%)	82% of patients assigned low risk	69% (±11%)
2 *N*=52	• Increased CD56 • CD34 and CD117 negative	Prevalence of 11q23 rearrangement (69%)	94% of patients assigned standard risk	39% (±14%)
3 *N* = 16	• Increased CD56 (very bright) • HLA-DR, CD45, and CD38 negative	Prevalence of *CBFA2T3–GLIS2* fusion transcript (63%)	100% assigned standard risk	19% (±20%)



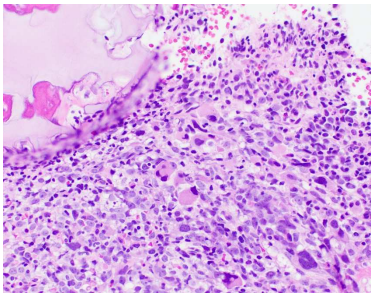
Cytometry B Clin Cytom. 2020 Jan; 98(1): 52–56.

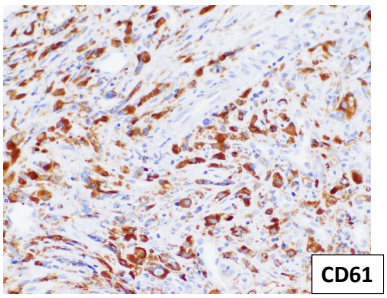
AML with RAM phenotype



AML with megakaryocytic differentiation

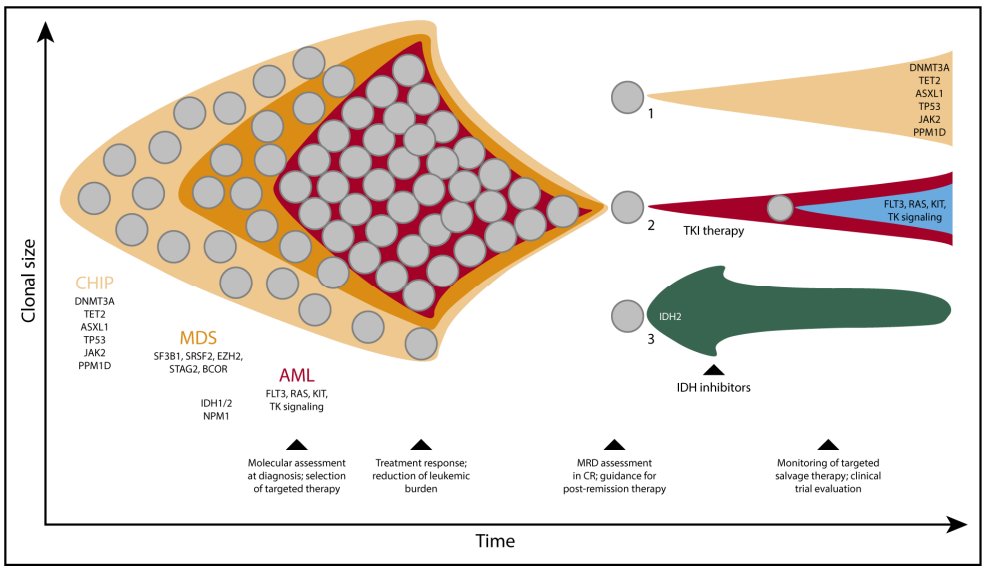
- 10% of pediatric AML patients and 1% of adult AML patients
- ≥20% bone marrow leukemic cells with at least 50% showing overt megakaryocytic lineage commitment
- Differential diagnosis includes
  - Myeloid leukemia associated with down syndrome and/or TAM
  - AML with t(1;22) (RBM15-MKL1)



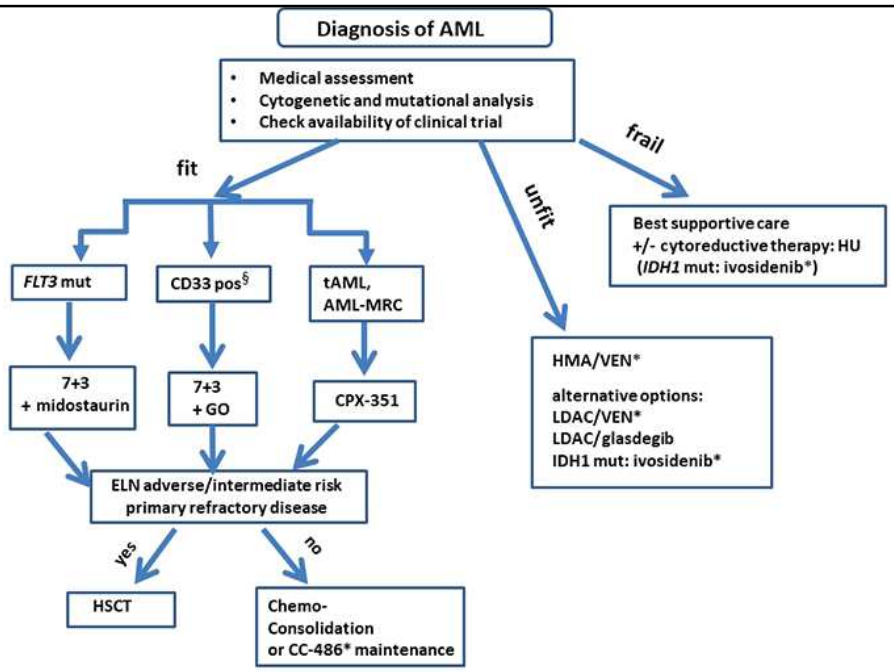


CD61

When to obtain genomic data in AML and which mutations matter



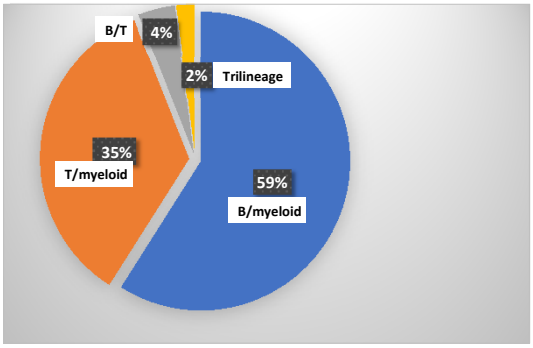
Blood Adv (2018) 2 (21): 3070–3080



Hematology Am Soc Hematol Educ Program (2021) 2021 (1): 16–23

Mixed Phenotype Acute leukemia (MPAL) Incidence

- represent ~2-3% of acute leukemias (SEERS- 0.35 cases per 1,000,000 person-years)
- M>F, peaks ≤19 and ≥60



Blast Lineage Requirements for Leukemias of Ambiguous Lineage

- *Myeloid*
  - Myeloperoxidase, or
  - Monocytic differentiation (2 or more: NSE, CD11c, CD14, CD64, lysozyme)
- *T lineage*
  - Cytoplasmic or surface CD3
- *B lineage*
  - Strong CD19 plus strong expression of at least 1 of CD79a, cCD22, CD10, or
  - Weak CD19 plus strong expression of at least 2 of CD79a, cCD22, CD10

Mixed Phenotype Acute Leukemia

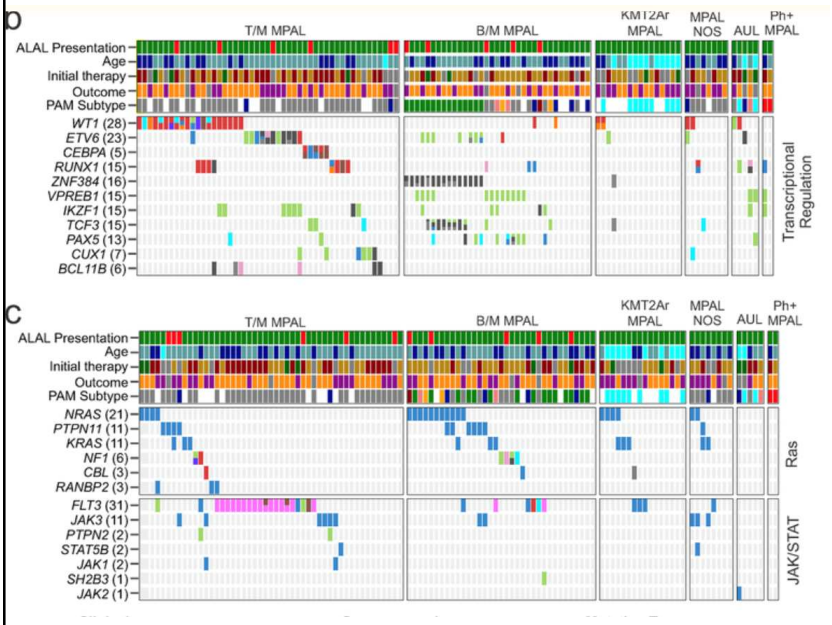
- With t(9;22)(q34;q11.2); *BCR-ABL1*
- With t(v;11q23); *MLL* rearranged
- B/myeloid, NOS
- T/myeloid, NOS
- NOS, rare types
  - T/B
  - T or B/megakaryocyte
  - T or B/erythroid

Cytogenetics of MPAL

- t(9;22)/(Ph) (20%)- D/D CML blast crisis (190 versus 210 kDa)
- 11q23/KMT2A- 8% - most frequently in infants
- complex 32%
- aberrant 27%
- Normal 13%.

→ ? AML-MRC or MPAL

Genetic basis of MPAL



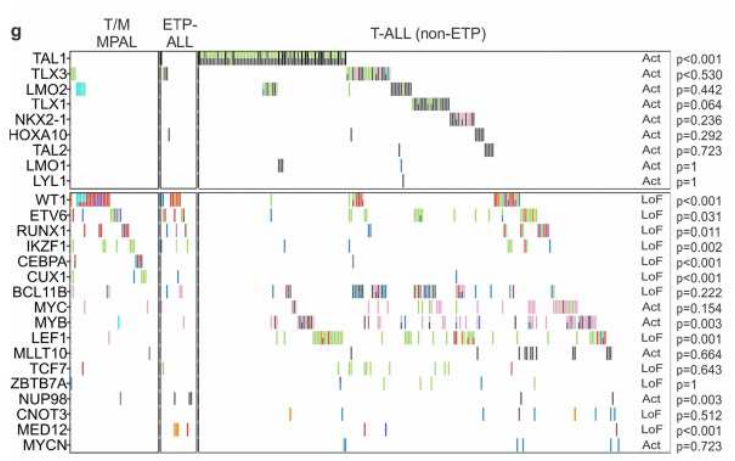
159 cases of pediatric ALAL
Exome (n=92), transcriptome (n=95), whole genome (n=47) sequencing
SNP array (n=95)

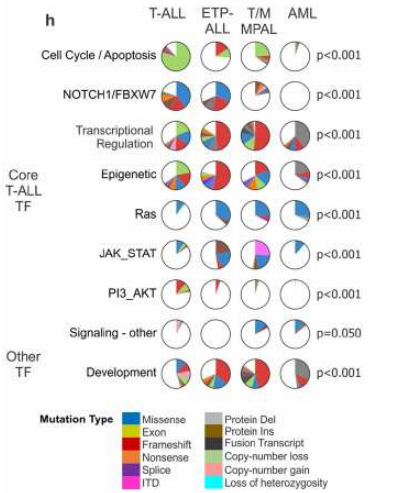
158 recurrently altered genes

Nature. 2018 Oct; 562(7727): 373–379.

ETP ALL vs T-Myeloid MPAL

Early T-cell Precursor (ETP) ALL: CD3+, CD1a-, CD8-, CD5- (or dim), expression of myeloid/stem cell antigens
T-Myeloid MPAL: CD3+ and requires MPO





Nature. 2018 Oct; 562(7727): 373–379

Mixed Phenotype Acute Leukemia - Leukemia blasts express specific antigens from multiple leukocyte lineages

***ZNF384* rearranged** - Leukemia blasts express antigens from multiple lineages
AND the presence of a *ZNF384* rearrangement

Ph-like - Leukemia blasts express antigens from multiple lineages
AND the presence of a Ph-like gene expression profile

***KMT2A* rearranged** - Leukemia blasts express antigens from multiple lineages
AND the presence of a *KMT2A* rearrangement

***BCR-ABL* positive** - Leukemia blasts express antigens from multiple lineages
AND the presence of a *BCR-ABL* fusion

T/myeloid, with *WT1* mutations - Leukemia blasts express both T-lymphoid and myeloid antigens
AND the presence of a *WT1* mutation

B/myeloid, NOS - Leukemia blasts express both B-lymphoid and myeloid antigens without a recurrent genetic abnormality

T/myeloid, NOS - Leukemia blasts express both T-lymphoid and myeloid antigens without a recurrent genetic abnormality

Not otherwise specified - Leukemia blasts express both B and T-lymphoid antigens
OR T-lymphoid, B-lymphoid, and myeloid antigens without a recurrent genetic abnormality

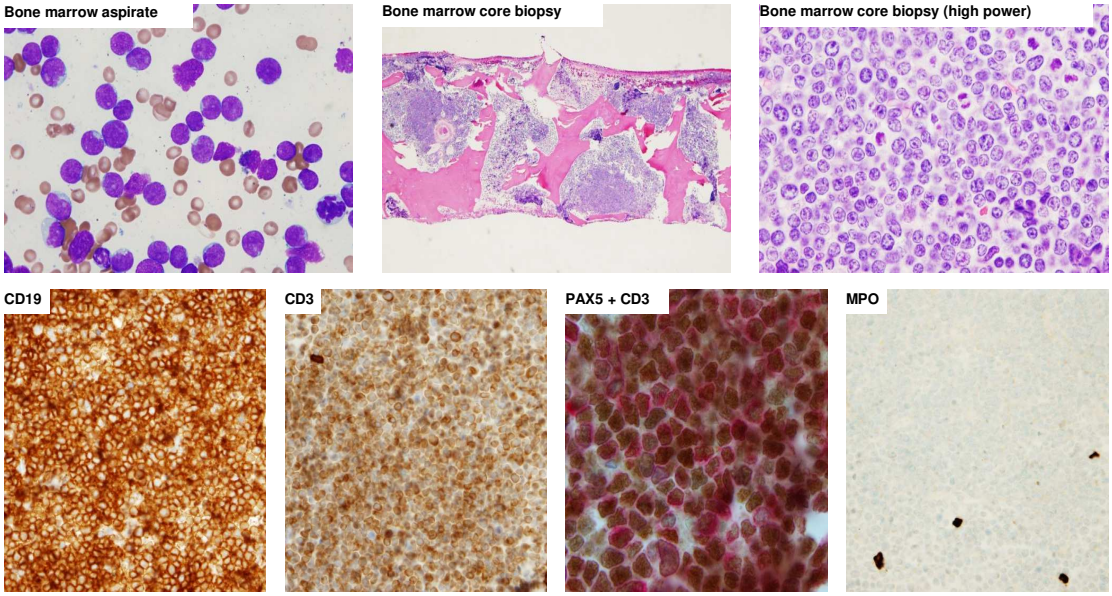
Acute Undifferentiated Leukemia - Leukemia blasts do not express any lineage defining antigens

Ph-like - Leukemia blasts do not express any lineage defining antigens
AND the presence of a Ph-like gene expression profile

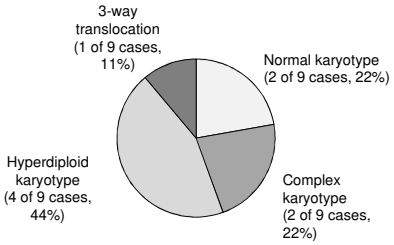
***KMT2A* rearranged** - Leukemia blasts do not express any lineage defining antigens
AND the presence of a *KMT2A* rearrangement

Not otherwise specified - Leukemia blasts do not express any lineage defining antigens
AND there is no recurrent genetic abnormality

B/T MPAL

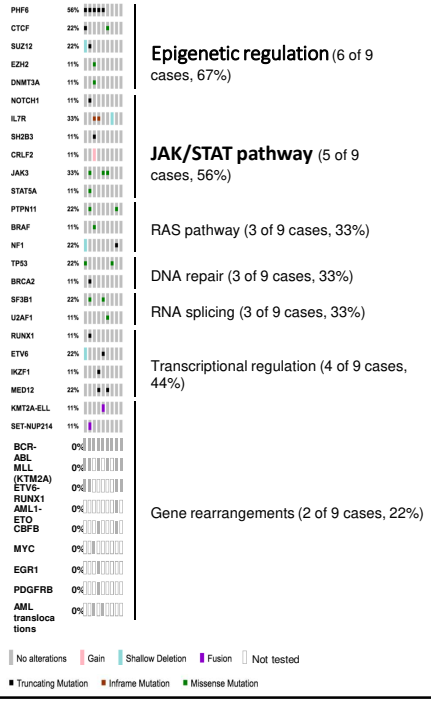


Am J Hematol. 2018 Nov;93(11):1358-1367



Patient	Karyotype
1	46,XX,-5,add(7)(q11.2),add(12)(p11.2),-13, del(20)(q11.2),+r[16],+mar1[cp20]*
2	46,XY[20]
3	46,XX[20]
4	47,XX,+11[14]/46,XX[6]
5	51,XX,+8,+10,+11,+13,+19[16]/46,XX[4]
6	50,XY,dup(1)(p22p36.1),+4,+10,-15, +21, +22, +mar[12]/50,idem,del(11)(q12),add(19)(q13.1)[3]/46,XY[5]
7	50,XY,+4mar[16]/46,XY[4]
8	46,XY,t(1;5;9)(p32;q3?3;p22)[6]/46,XY[17]**
9	47,XY,+8,del(12)(p12)[8]/47,idem,del(9)(q?34)[4]/46,XY[8]

Am J Hematol. 2018 Nov;93(11):1358-1367



NK Lymphoblastic leukemia

- NK-lymphoblastic leukemia has been difficult to define; only rare case reports
- Varying terminology has been used over the years
  - Myeloid/NK acute leukemia
    - Overlap with AML with minimal differentiation
  - Blastic NK cell lymphomas/leukemias
    - Now recognized as blastic plasmacytoid dendritic cell neoplasm (BPDCN)
- Considered as *provisional entity* in WHO classification
  - Expression of CD56, CD7 and CD2, and cCD3
  - Absence of B-cell and myeloid markers
  - TCR and IG genes are in the germline configuration

Blood 2016;127(20):2391-405
Leukemia & Lymphoma, 2002;43(4): 901-906

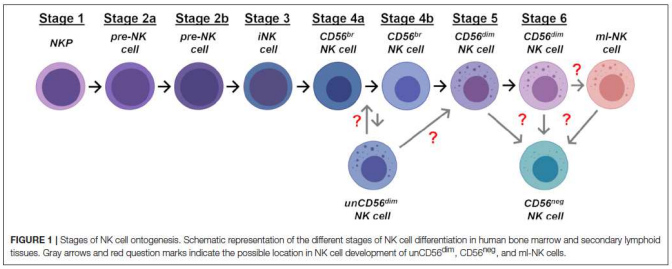


FIGURE 1 | Stages of NK cell ontogenesis. Schematic representation of the different stages of NK cell differentiation in human bone marrow and secondary lymphoid tissues. Gray arrows and red question marks indicate the possible location in NK cell development of unCD56^{dim}, CD56^{neg}, and ml-NK cells.

TABLE 1 | Principal surface markers differentially expressed on NK cell developmental intermediates.

Surface marker	Stage 1	Stage 2a	Stage 2b	Stage 3	Stage 4a	Stage 4b	Un CD56^{dim}	Stage 5	Stage 6	ml-NK	CD56^{neg}
CD34	+	+	+	–	–	–	–	–	–	–	–
CD10	+	+/–	+/–	–	–	–	n.d.	–	–	–	–
HLA-DR	+	+	+	–	–	–	n.d.	–	–	+	+
CD117	–	+	+	+	+/low	low/–	–	–	–	–	–
CD127	+	+	+	+	–	–	–	–	–	–	+
CD45RA	+	+	+	+	+/–	+/–	n.d.	–	–	–	–
IL-1 βR	–	–	+	+	+/low	low/–	n.d.	low/–	low/–	low/–	–
CD122	–	–	+	+	+	+	n.d.	+	+	+	+
CD161	–	–/low	–/low	–/+	+	+	n.d.	+	+	low/–	+
CD56	–	–	–/low	–/low	++	++	+	+	+	+	–
CD94	–	–	–	–	+	+	+	+/–	+/–	+	+
NKG2A	–	–	–	–	+	+	+	low/–	low/–	low/–	low/–
NKG2D	–	–	–	–/low	+	+	+	+	+	+	+
NKp30	–	–	–	–/low	+	+	+	+	+	low/–	low
NKp46	–	–	–	–/low	++	++	–/low	+	+	low	low
NKp80	–	–	–	–	–	+	n.d.	+	+	+	+
NKG2C	–	–	–	–	low/–	low/–	low/–	+	+	++	+/low
CD16	–	–	–	–	–	–	–	+	+	+	+
KIRs	–	–	–	–	–	–	low	+	+	+	+
CD57	–	–	–	–	–	–	–	–	+	+	+

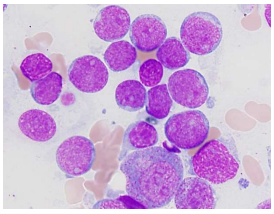
n.d., not determined.

- Early NK cells mature in bone marrow and show non-specific patterns of early marker expression
  - More specific NK markers such as CD161, CD127 and CD94 are not commonly tested

Front Immunol 2019 Aug 2;10:1812

Recent NK-LL case series

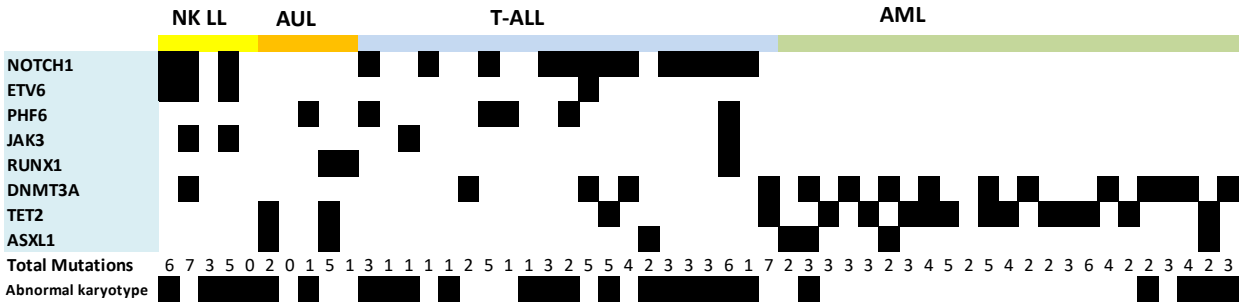
- Identified 6 cases of NK lymphoblastic leukemia through a multi-institutional search with clinical, pathologic, molecular and outcome data
  - *NK LL defined using WHO classification*
    - *Expression of CD56, CD7 and CD2, and cCD3*
    - *Absence of B-cell and myeloid markers*
    - *TCR and IG genes are in the germline configuration*



- Compare with control CD56+ acute leukemias:
  - 6 cases of AUL, 51 cases of T-ALL (14 ETP), 42 cases of AML
- NK-LL patients were significantly younger and presented with higher WBC and platelets
- Immunophenotypic differences
  - frequent expression of cytoplasmic CD3 and CD33 in NK-LL as compared to AUL.
  - Compared to T-ALL, NK-LL cases showed less frequent cCD3, CD4, and CD10
  - NK-LL patients showed brighter CD56 expression as compared to ETP-ALL patients and less frequent cytoplasmic CD3
- No Difference in rates of abnormal karyotypes between all 4 groups

Mod Pathol 2021 Jul;34(7):1358-1366

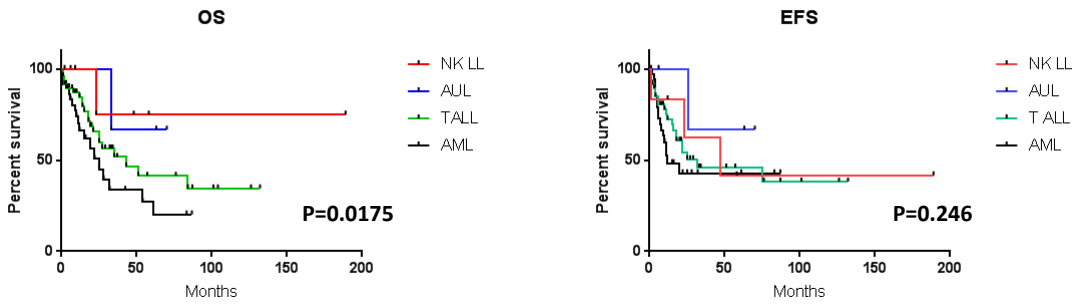
Mutational Profile



- Significant **enrichment** :
  - *NOTCH1* mutation in NK LL as compared to AML (p=0.002)
  - *ETV6* mutations in NK LL as compared to T-ALL (p=0.004) and AML (p=0.0022)
  - *JAK3* in NK LL as compared to AML (p=0.02)
- Significant **absence** :
  - *TET2* mutation in NK LL as compared to AML (p=0.05)
- **Total mutations** are significantly higher in NK LL as compared to T-ALL (5 vs 2, p=0.04)

Mod Pathol 2021 Jul;34(7):1358-1366

Clinical Outcome



- All 6 NK LL patients treated with **ALL type therapy upfront** while 5/6 AUL patients treated with AML therapy
- BMT: 1/6 NK LL, 3/6 AUL, 12/51 T-ALL patients, 16/42 AML patients

Mod Pathol 2021 Jul;34(7):1358-1366

Acute Undifferentiated Leukemia (AUL)

- Rare type of acute leukemia that shows no evidence of differentiation along any lineage
- Little is known about AUL, including the optimal number and types of myeloid markers allowed in this diagnosis.

- AML with minimal differentiation is a subtype of AML, not otherwise specified (NOS) in the WHO classification and roughly correlates with **AML-M0** in the French-American-British classification.
  - **5% or less of all AML cases**, is by definition negative for myeloperoxidase, and expresses at least two myeloid marker, usually CD13, CD33 and/or CD117

- *Clinical, immunophenotypic and genetic data is limited and it is uncertain if AUL is biologically distinct from AML with minimal differentiation/AML M0*

AUL multi-institutional study

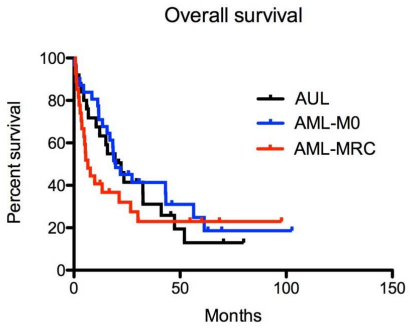
- 89 cases from 8 academic institutions identified on search AUL or AML M0/AML minimal differentiation
  - 31 are re-classified as AML MRC on basis of having complex/MDS type of karyotype [**excluded from AUL**]
  - 27 AUL and 30 AML M0/AML minimal differentiation
- 6 AUL cases (22%) showed **no myeloid marker expression** (CD117, CD13 or CD33)
  - 15 AUL cases (55%) showed partial or full expression of **1 myeloid marker**
  - 3 (11%) showed expression of **1 myeloid marker +** weak/partial expression of another myeloid marker on the blasts

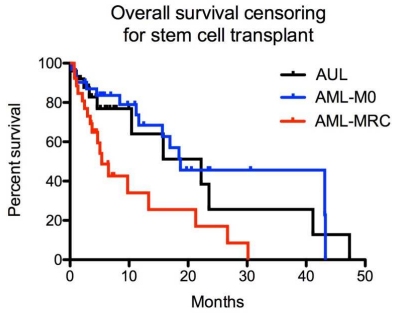
Weinberg O Mod Pathol. 2019 Sep;32(9):1373-1385.

AUL multi-institutional study

- Immunophenotype: TdT and CD123 more prevalent in AUL
  - None of the cases expressed cytoplasmic or surface CD3, but expression of other T-cell associated antigens was commonly seen on blasts (11/24, 46%)
- Fourteen AUL cases (58%) had a normal karyotype, and of the abnormal karyotypes, 5 had trisomy 13 (55%)
- Molecular: *PHF6* mutations more frequent in AUL (5/15 vs 0/19, p = 0.016)
  - Re-assigning cases with expression of second myeloid markers to AML shows more signficant association with ***PHF6 mutations*** (5/13 vs 0/21, $p = 0.0046$).
- Other frequent mutations included *SRSF2, RUNX1, ASXL1* and *DNMT3A*

Weinberg O Mod Pathol. 2019 Sep;32(9):1373-1385.



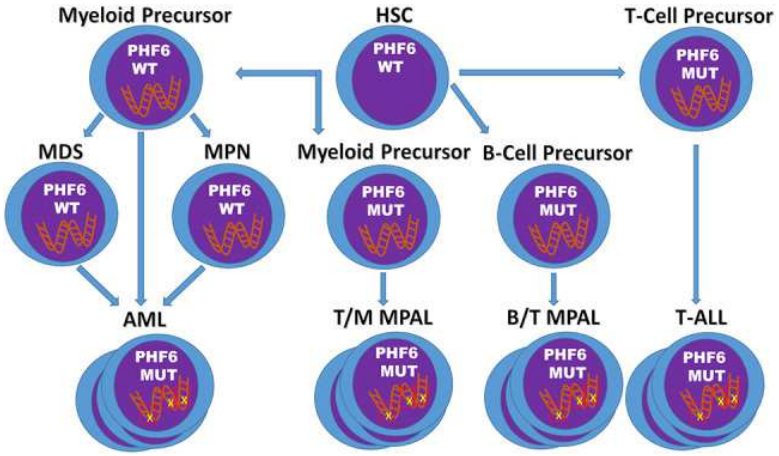


- 27 AUL patients presented with similar age, blood counts, bone marrow cellularity, and blast percentage as the 31 AML MD patients
- Most AUL patients were treated with AML type therapy with ~50% achieving complete remission
- 31 AML MRC patients showed high frequency of complex karyotype and *TP53* mutations

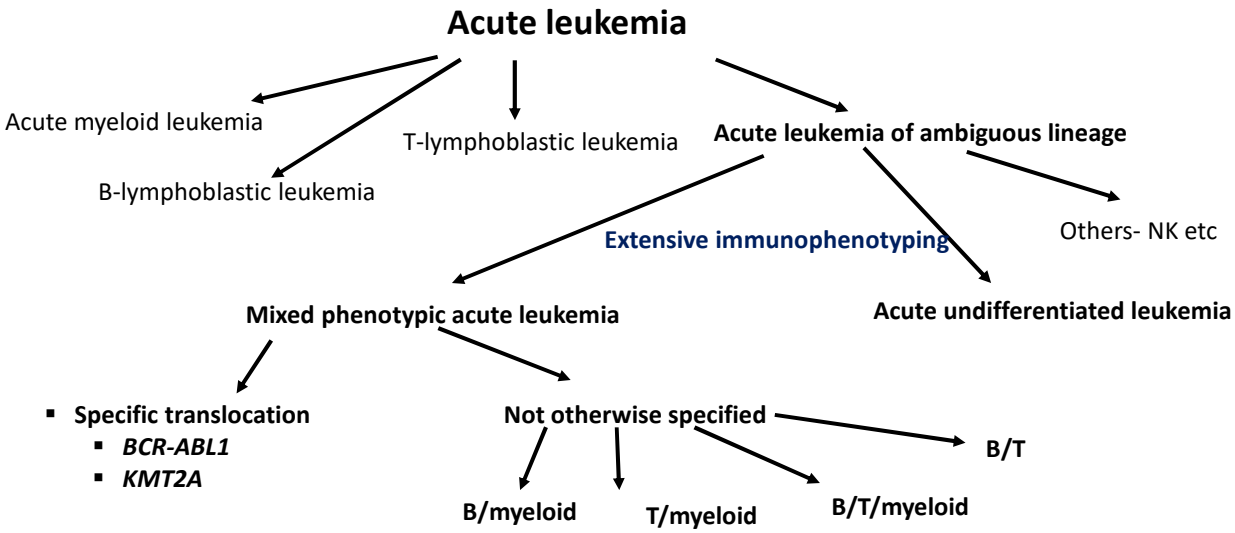
Weinberg O Mod Pathol. 2019 Sep;32(9):1373-1385.

Model of *PHF6* in Hematopoietic Malignancies

The timing and context of the acquisition of *PHF6* deletions/mutations appear to determine the fate of the resulting malignancy.



Front Oncol 2021 Jul 26;11:704471. doi: 10.3389/fonc.2021.704471



Conclusions

- Large-scale sequencing studies of hematologic malignancies have revealed numerous genetic findings in myeloid neoplasms with some correlations with phenotype
- Mixed-phenotype acute leukemia is a diagnostic and therapeutic challenge owing to its heterogeneity, overlapping features with other types of ALL and AML, and lineage plasticity.
- Several unresolved questions about the diagnostic criteria for MPAL that will require further studies of the correlation between immunophenotype, genotype, lineage plasticity, and therapeutic response

Thank you!!